

CLAIMS

1. A bacterial autoinducer, characterised in that it has substantially the following properties:

- Sub-B8
- i) it is produced in response to noradrenaline in serum SAPI medium;
  - ii) it is heat stable;
  - iii) it is stable to lyophilisation;
  - iv) it has a negative charge;
  - v) it is polar;
  - vi) it is hydrophilic;
  - vii) it will not partition into organic solvents;
  - viii) it is capable of binding positively charged metal ions; and
  - ix) it has a molecular weight of about 300-1500 daltons

2. A bacterial autoinducer according to claim 1, further characterised in having at least one of the following characteristics:

- i) it has absorbion maxima at 255,325 and 500-550nm; and
- ii) it is stable in prolonged storage in a dried state and/or in solution.

3. A bacterial autoinducer according to <sup>claim 1</sup> ~~either one of claims 1 or 2~~, further characterised in having at least one of the following characteristics:

- i) it is produced in substantially smaller quantities by bacteria grown in LURIA broth, Tryptone soya broth, M9 minimal medium and Davis-Mingioli/minimal medium than by the same bacteria grown in serum SAPI medium;
- ii) it has a reddish-pink colour, reversibly decolorisable by reducing the pH to <4;
- iii) it contains serine;
- iv) its synthesis involves the entA and entB gene products;

- Sub.B8
- v) its synthesis is not stimulated by conditions of Fe starvation;
  - vi) it is synthesised in conditions of excess Fe;
  - vii) its entry into bacteria occurs via a tonB dependent receptor;
  - viii) it is inactivated by oxidation;
  - ix) it is inactivated by extreme pH; and
  - x) it is resistant to degradation by ribonuclease, deoxyribonuclease, trypsin, pepsin, V8 protease, proteinase K, acid phosphatases, alkaline phosphates and phosphodiesterase.

4. A bacterial autoinducer according to <sup>claim 1</sup> ~~any one of the preceding claims~~, being an *E.coli*, *Hafnia alvei* or *Salmonella* autoinducer

5. A method for isolating and purifying a bacterial autoinducer, comprising the steps of:

- i) collecting a sample containing the autoinducer;
- ii) fractionating the sample to isolate fractions corresponding to molecular weights of approximately 300-1500 Daltons; and
- iii) eluting the isolate of (ii) on an anion-exchange chromatographic column and selecting the fraction containing the autoinducer.

6. A method according to claim 5, comprising the additional step of concentrating the sample prior to fractionating.

7. A method according to claim 6, concentration being achieved by passing the sample through an approximately 0.2  $\mu\text{m}$  diameter filter, lyophilising the sample and passing it through an approximately 0.2  $\mu\text{m}$  diameter filter.

8. A method according to claim 6, concentration being achieved by means of ultrafiltration.

9. A method according to claim 8, ultrafiltration being performed with a molecular weight cut-off of approximately 100 Daltons.

G 10. <sup>Sub B9</sup> A method according to <sup>claim 5</sup> ~~any one of claims 5-9~~, the sample being collected from a culture containing bacteria and the autoinducer.

11. A method according to claim 10, the sample being a supernatant collected from a centrifuged culture containing bacteria and the autoinducer.

Q 12. <sup>Sub B10</sup> A method according to <sup>claim 5</sup> ~~any one of claims 5-11~~, size exclusion gel filtration being performed using a buffer of approximately 100 mM ammonium bicarbonate, pH 8.0, anion exchange purification being performed on an anion exchange column and triethylammonium bicarbonate.

A 13. <sup>claim 5</sup> A method according to ~~any one of claims 5-11~~, size exclusion gel filtration being performed using a buffer of approximately 20 mM potassium phosphate containing 150 mM NaCl, pH 7.4, anion exchange purification being performed on an anion exchange column and NaCl gradient.

G 14. <sup>claim 5</sup> A method according to ~~any one of claims 5-13~~, the bacterium from which the autoinducer is derived being *E. coli*, *Salmonella* or *Hafnia alvei*.

15. <sup>claim 5</sup> A bacterial autoinducer isolated and purified according to the method of ~~any one of claims 5-14~~.

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Parameter	Estimate	Standard Error	t-Statistic	p-Value	95% Confidence Interval
Intercept	1.0000	0.0000	1.0000	1.0000	1.0000
Age	0.0000	0.0000	0.0000	1.0000	0.0000
Gender	0.0000	0.0000	0.0000	1.0000	0.0000
Education	0.0000	0.0000	0.0000	1.0000	0.0000
Income	0.0000	0.0000	0.0000	1.0000	0.0000
Health	0.0000	0.0000	0.0000	1.0000	0.0000
Marital Status	0.0000	0.0000	0.0000	1.0000	0.0000
Religion	0.0000	0.0000	0.0000	1.0000	0.0000
Occupation	0.0000	0.0000	0.0000	1.0000	0.0000
Region	0.0000	0.0000	0.0000	1.0000	0.0000
Time	0.0000	0.0000	0.0000	1.0000	0.0000
Constant	1.0000	0.0000	1.0000	1.0000	1.0000